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Dynamics of Oligodendrocyte Generation and Myelination in the Human Brain

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SUMMARY

The myelination of axons by oligodendrocytes has been suggested to be modulated by experience, which could mediate neural plasticity by optimizing the performance of the circuitry. We have assessed the dynamics of oligodendrocyte generation and myelination in the human brain. The number of oligodendrocytes in the corpus callosum is established in childhood and remains stable after that. Analysis of the integration of nuclear bomb test-derived ^{14}C revealed that myelin is exchanged at a high rate, whereas the oligodendrocyte population in white matter is remarkably stable in humans, with an annual exchange of 1/300 oligodendrocytes. We conclude that oligodendrocyte turnover contributes minimally to myelin modulation in human white matter and that this instead may be carried out by mature oligodendrocytes, which may facilitate rapid neural plasticity.

INTRODUCTION

Oligodendrocytes wrap layers of specialized cell membrane around nerve fibers to form myelin, which provides electrical insulation to increase axonal conduction velocity and the speed of neural processing. Myelination is a largely postnatal process and it continues well into adulthood in humans. Practicing a skill can increase the volume of white matter regions employed in carrying out the task and, conversely, social isolation, with reduced external stimuli, leads to hypomyelination and impaired cognitive functions (Blumenfeld-Katzir et al., 2011; Gibson et al., 2014; Liu et al., 2012; Makinodan et al., 2012; Sampaio-Baptista et al., 2013). This has led to the suggestion that external stimuli may modulate myelination, which in turn could affect axonal transmission velocity and neural processing (Bergmann and Frisén, 2013; Fields, 2008, 2012).

How myelin is resculpted during brain maturation and in response to experience is not fully understood. Myelination can in theory be modified by mature oligodendrocytes and/or by exchanging oligodendrocytes and their myelin sheaths. Several observations support that oligodendrocyte turnover contributes to myelin remodeling. Oligodendrocyte progenitor cell proliferation and initiation of myelination has been suggested to be regulated by neural activity (Barres and Raff, 1993; Demerens et al., 1996; Fields, 2012; Gibson et al., 2014; Wake et al., 2011) and myelinating oligodendrocytes continue to be generated in adulthood at a substantial rate in rodents (Barnabé-Heider et al., 2010; Dimou et al., 2008; Emery, 2010; Rivers et al., 2008; Young et al., 2013). It is less clear to what extent mature oligodendrocytes can modulate their myelination. Whereas transplantation of oligodendrocyte progenitors to the central nervous system results in the differentiation of new oligodendrocytes that readily myelinate axons after transplantation, transplanted mature oligodendrocytes fail to myelinate (Blakemore and Keirstead, 1999). Moreover, newly differentiated rodent oligodendrocytes can myelinate axons in vitro, whereas mature oligodendrocytes do so only very inefficiently (Watkins et al., 2008). Imaging of myelination by rodent cells in vitro and in vivo during zebrafish development have demonstrated that oligodendrocytes establish all their myelin segments within a few hours after terminal differentiation and there appears to be little plasticity in terms of generating new myelin sheaths after that time window (Czopka et al., 2013; Watkins et al., 2008). However, manipulation of certain signaling pathways in mouse oligodendrocytes results in enhanced myelination and a larger number of myelin layers, indicating that the thickness of the myelin sheath may be dynamically modulated (Flores et al., 2008; Goebbels et al., 2010; Snaidero et al., 2014). Thus, available data from model organisms indicate that the number of myelin segments of a mature oligodendrocyte is static, but that oligodendrocyte turnover and potentially modulating the thickness of myelin sheaths contributes to myelin remodeling.

We have assessed the dynamics of oligodendrocyte generation and myelination in humans. The number of oligodendrocytes in the corpus callosum is established in childhood and remains

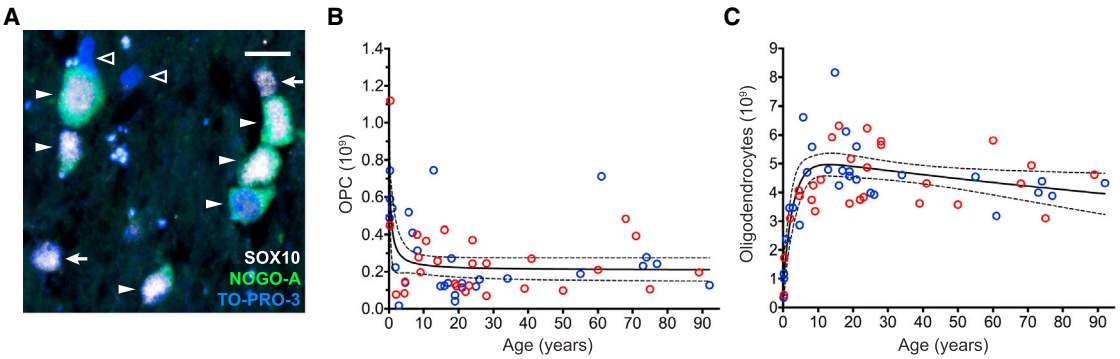

Figure 1. Oligodendrocyte Generation in the Human Corpus Callosum

(A) Oligodendrocyte progenitor cells (OPC, SOX10+, NOGO-A−, arrows) and mature oligodendrocytes (SOX10+/NOGO-A+, solid arrowheads) were identified in histological sections from the human postmortem corpus callosum and quantified by stereology. Immature oligodendrocyte lineage cells lack NOGO-A and nonoligodendrocyte lineage cells also lack SOX10 (hollow arrowheads). Cell nuclei are visualized with TO-PRO-3. Scale bar represents 10 μm.

(B and C) The number of oligodendrocyte progenitor cells (B) and oligodendrocytes (C) in the human corpus callosum. Males are indicated with blue circles and females with red circles. The solid lines represents a double exponential fitting of the data (see also Extended Experimental Procedures) and the dashed lines represent 95% confidence bands.

See also Figure S1 and Tables S1 and S2.

stable after that. The oligodendrocyte population in human white matter is remarkably static once the full complement is established, with only 1/300 oligodendrocytes being exchanged annually, and oligodendrocyte generation cannot account for the increase in myelin volume in response to experience in humans. We conclude that myelin remodeling in white matter is independent of cell turnover and mainly carried out by mature oligodendrocytes in humans.

RESULTS

The Number of Oligodendrocytes in the Corpus Callosum Is Established in Childhood

We first set out to establish the time course of oligodendrocyte generation in humans. We focused on the corpus callosum, the largest commissure, which is easily delineated anatomically. The corpus callosum represents a white matter tract that is modulated by experience throughout life (Bengtsson et al., 2005; Lövdén et al., 2010) and the integrity of the corpus callosum white matter correlates with the performance of this neural circuitry in humans (Johansen-Berg et al., 2007).

We used quantitative stereology to establish the number of oligodendrocyte progenitors and mature oligodendrocytes in the human postmortem corpus callosum in subjects aged 0.2–92 years (n = 55). Mature oligodendrocytes were identified by their combined expression of SOX10, a general oligodendrocyte lineage marker (Stolt et al., 2002), and NOGO-A, a marker for mature myelinating oligodendrocytes (Schwab, 2010). We found that 99.2% of corpus callosum cells that expressed NOGO-A were positive for adenomatous polyposis coli (APC) (n = 1,496 cells) (Bhat et al., 1996) and 99.5% were positive for Myelin basic protein (n = 745 cells), both commonly used markers for mature myelinating oligodendrocytes, validating that NOGO-A can be used to accurately identify mature oligodendrocytes in this situation. SOX10+/NOGO-A− cells were defined as oligodendrocyte progenitor cells (Figure 1A and Figure S1 available online).

The number of oligodendrocyte progenitors was highest in the youngest individuals and dropped during early childhood to approach stable numbers at ~5 years of age (Figure 1B). Shortly after birth, there were very few mature oligodendrocytes, but the number increased rapidly in the perinatal period. The number of oligodendrocytes started to plateau at ~5 years of age (88% of the final number). The sterological data indicated that 98.5% of the final number of oligodendrocytes was reached at 9 years of age. After this age the number of oligodendrocytes stayed largely stable throughout the rest of the human lifespan (Figure 1C; Tables S1 and S2).

Dynamics of Myelination in Humans

Measurements of white matter volume by magnetic resonance imaging have established a continuous increase into early adulthood (Lebel et al., 2012). It is, however, not possible to selectively visualize myelin by imaging, and the change in volume will also be influenced by for example changes in axon number, axon diameter, and the number of glial cells. To establish the dynamics specifically of myelination, we biochemically isolated myelin from the human postmortem corpus callosum from subjects aged 0.2–92 years (n = 57, including all subjects in whom we had quantified the number of oligodendrocytes, Figure 1C) and measured the volume (Figure S2A). This revealed an initially steep increase in myelin volume (86% of the final volume was reached at 5 years of age). The increase continued into adolescence, reaching its maximum at 17 years of age. This was followed by a slow gradual decline during aging, largely paralleling the total volume of the corpus callosum established by imaging (Figures 2A and S2B; Tables S3 and S4).

There was substantial interindividual variation in corpus callosum myelin volume (coefficient of variation, CV = 48.3%, n = 57 subjects) and the number of oligodendrocytes (CV = 31.3%, n = 55), as well as the total volume of the corpus callosum, established by MRI (CV = 22.7%, n = 403). In contrast to the sexual

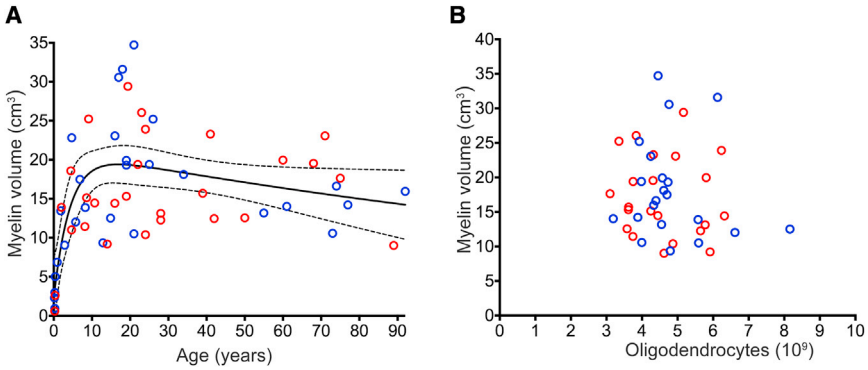

Figure 2. Myelination of the Human Corpus Callosum

(A) The myelin volume in the corpus callosum of the same subjects as in Figures 1B and 1C. The solid line represent double exponential curve fitting and the dashed lines represent 95% confidence bands.

(B) There is no correlation between the number of oligodendrocytes and the myelin volume in the corpus callosum (Pearson's correlation, $r = -0.10$, $p = 0.52$) once the final complement of oligodendrocytes is established. Data for individuals >5 years of age are shown. Males are indicated in blue and females in red.

See also Figure S2 and Tables S3 and S4.

dimorphism in oligodendrocyte generation and myelin gene expression in rodents (Cerghet et al., 2006), there was no statistically significant difference in the number of oligodendrocytes or myelin volume between men and women ($p = 0.95$ and $p = 0.70$, respectively, Mann-Whitney U test). After the age of 5, when the number of oligodendrocytes started to plateau (Figure 1C), there was no correlation between the number of oligodendrocytes and the myelin volume (Figure 2B, Pearson's correlation, $r = -0.10$, $p = 0.52$). Thus, the number of oligodendrocytes does not appear to be a major determinant of the myelin volume in humans after the initial expansion phase.

Turnover of Myelin and White Matter Cells in Humans

White matter volume, and likely myelination, can increase several percent within a few weeks in humans in response to practicing a skill (Scholz et al., 2009). If changes in myelin volume in response to experience is mediated by newly generated oligodendrocytes, rather than by preexisting mature oligodendrocytes modulating their myelination, a substantial proportion of the oligodendrocytes would need to be exchanged.

To assess the cell turnover dynamics in human brain white matter, we retrospectively birth-dated corpus callosum cells by analyzing the integration of nuclear bomb test-derived ^{14}C by accelerator mass spectrometry (Spalding et al., 2005) (Figure 3A). The ^{14}C concentration in genomic DNA in cells from the human postmortem corpus callosum (n = 12 subjects), as well as frontal lobe white matter (n = 3), corresponded to the atmospheric levels within a few years after the birth of the individuals in subjects born after the nuclear bomb tests (Figure 3B; Table S5), indicating very limited cell turnover. In individuals born before the onset of the nuclear bomb tests, the ^{14}C levels in genomic DNA were lower than at any time after 1955 (Figure 3B), establishing that a large proportion of cells in white matter had not been exchanged for more than 5 decades.

We also measured the concentration of ^{14}C in biochemically purified myelin (Table S5). In all individuals (n = 10), the ^{14}C concentration in myelin corresponded to the atmospheric ^{14}C concentration around the time of death of the subject (Figure 3C), demonstrating that myelin is contemporary and continuously exchanged in humans. This is in line with data from rodents, where it has been established that myelin proteins are comparatively stable proteins, albeit exchanged within months (Savas et al., 2012; Toyama et al., 2013). We cannot distinguish whether this reflects exchange of myelin sheaths or molecular turnover in stable myelin sheaths.

Isolation of Oligodendrocyte Nuclei from the Human Postmortem Brain

Although oligodendrocytes are in majority in white matter tracts (75.4% ± 5.1% of cells in the human corpus callosum, mean ± SD, n = 22 subjects and 78.4% ± 2.2%, n = 6, in the frontal lobe, as assessed by flow cytometry, see below), it was important to specifically isolate and birth date oligodendrocytes, as it is likely that the turnover dynamics of diverse cell populations may be different. We developed a strategy to isolate oligodendrocyte nuclei by flow cytometry. We isolated SOX10+/APC+ nuclei from the human postmortem corpus callosum by flow cytometry from 34 subjects aged 4–92 (Figure 4A). The number of oligodendrocytes and the myelin volume was determined in all except three of these cases (shown in Figures 1 and 2). Reanalysis demonstrated 98.2% ± 1.2% (mean ± SD, n = 34 subjects) purity of the SOX10+/APC+ population (Figure S3). The specificity of the isolation was further assessed by quantitative RT-PCR (qRT-PCR), which revealed that 95.3%–99.6% of the mRNA for three different mature myelinating oligodendrocyte markers and *SOX10* was present in the SOX10+/APC+ fraction (Figure 4B). Furthermore, the SOX10+/APC+ nuclear fraction was almost devoid of markers for astrocytes, microglia, oligodendrocyte progenitors, endothelial cells, and hematopoietic cells (Figure S3), establishing that the SOX10+/APC+ fraction consisted of highly enriched mature oligodendrocyte nuclei. Conversely, the nonoligodendrocyte fraction was highly enriched for markers for other cell types and largely depleted of markers for mature oligodendrocytes (Figures S3 and 4B) Thus, nearly all of the oligodendrocyte nuclei were isolated in the SOX10+/APC+ fraction.

Turnover of Oligodendrocytes in Humans

In individuals born before the onset of the nuclear bomb tests in 1955 (n = 10), the ^{14}C concentration in oligodendrocyte genomic DNA from the corpus callosum was higher than the prebomb atmospheric concentrations, demonstrating that oligodendrocytes had been generated after the onset of the ^{14}C increase in 1955 (Figure 5A; Table S5). However, in 9 out of 10 individuals, the ^{14}C concentration was lower than at any time after the onset of the nuclear bomb tests, establishing that a large proportion of

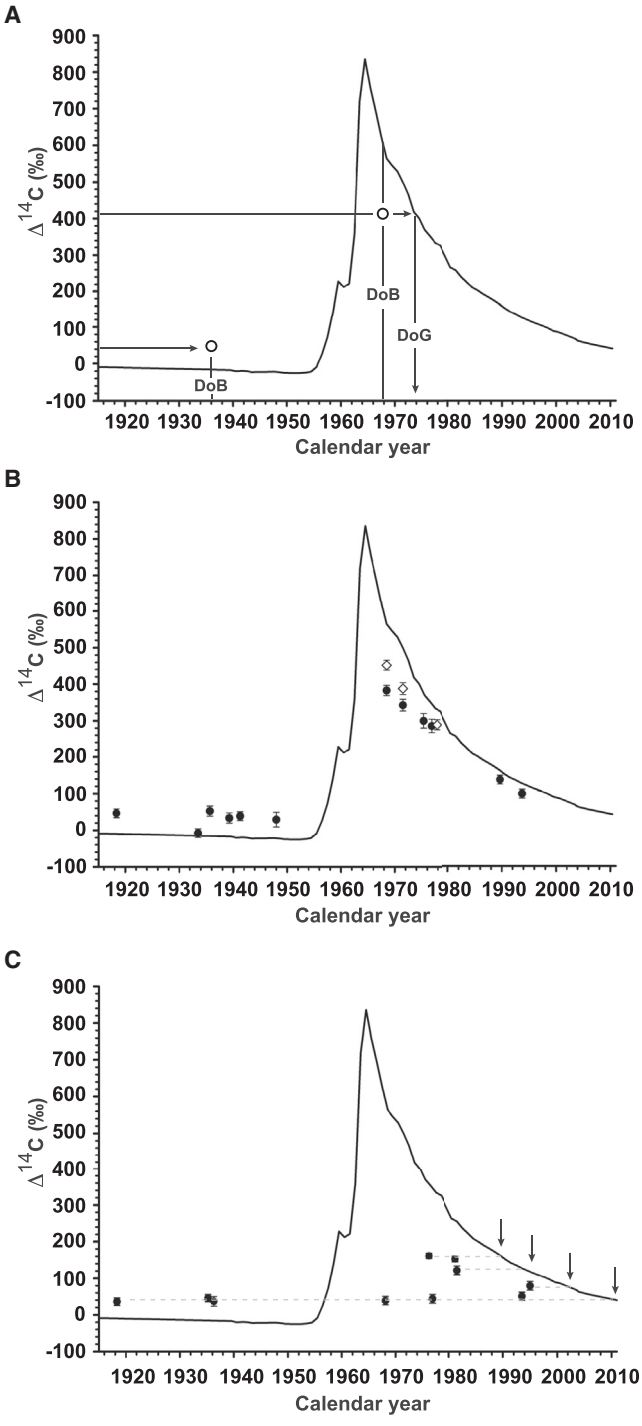

Figure 3. Myelin Is Young and White Matter Cells Are Old

(A) Schematic depiction of the presentation of ^{14}C data. The curve indicates the atmospheric ^{14}C concentration over time. The data (circles) is plotted based on the date of birth (DoB) of the person and the ^{14}C concentration in myelin or genomic DNA. For individuals born after the nuclear bomb tests (1955–1963), the date of generation (DoG) of cells or myelin can be inferred by reading of the x axis, whereas this cannot be directly inferred for subjects born before the onset of the increase in ^{14}C. If not otherwise stated, the date of death of the studied subjects was 2009–2012 in all figures.

the oligodendrocytes must have been generated before 1955 and lasted for more than 5 decades. Individuals born after the nuclear bomb tests had ^{14}C concentrations in oligodendrocyte DNA that corresponded to within a few years after the subjects' birth (Figure 5).

We also carbon dated genomic DNA from oligodendrocyte nuclei (SOX10+/APC+) isolated from frontal lobe white matter (n = 6 individuals), which revealed very similar ^{14}C concentrations to oligodendrocyte nuclei isolated from the corpus callosum (Figure S4A). The ^{14}C levels were very similar in oligodendrocyte DNA (Figures 5 and S4A) and unsorted white matter cell DNA (Figure 3B), indicating that there cannot be any sizable subpopulation of oligodendrocytes with very different turnover dynamics in these regions, which were excluded in our strategy for isolating oligodendrocyte nuclei.

We next carbon dated genomic DNA from oligodendrocytes isolated from prefrontal or frontal cortex gray matter in subjects aged 8–92 years (n = 20). Interestingly, individuals born before the onset of the nuclear bomb tests had higher and individuals born after the peak in atmospheric ^{14}C concentration had lower ^{14}C levels in gray matter oligodendrocytes compared to in white matter oligodendrocytes in the same subjects (Figures S4C and S4D). Isolated cortical and cerebellar neurons had ^{14}C concentrations corresponding to the time around their birth (Figure S4B), in line with the lack of any detectable postnatal neurogenesis in these regions (Bergmann et al., 2012; Bhardwaj et al., 2006; Spalding et al., 2005).

We assessed the expression of Ki67, a marker for cells in cycle, in oligodendrocyte lineage cells in the human corpus callosum (n = 6 subjects, age 0.3–13 years). Ki67-positive oligodendrocyte progenitors (SOX10+/NOGO-A− cells) were abundant in the early perinatal period but their number had decreased to very low levels by 4 years age, and such cells were difficult to find at later stages (Figures S4E–S4G). We next assessed the presence of the thymidine analog IdU in oligodendrocyte lineage cells in gray and white matter in postmortem tissue from frontal and occipital cortex from three patients (ages 17–51), who had received the compound as a radiosensitizer. Although sparse IdU-labeled cells were present in the brain parenchyma, IdU-labeled oligodendrocytes were extremely rare and we only identified one such cell (Figure S4H) when analyzing >10,000 mature oligodendrocytes in each patient. The very low numbers of oligodendrocyte progenitor cells in cycle after the perinatal stage, or that had been generated during a pulse of IdU administration, corroborated that there is very limited generation of oligodendrocytes after early childhood.

(B) The ^{14}C concentration in genomic DNA from corpus callosum (black circles) and frontal lobe white matter (white diamonds) cells corresponds to within a few years after the birth of individuals born after the nuclear bomb tests. In subjects born before the onset of the nuclear bomb tests, the ^{14}C concentration is lower than contemporary levels, indicating that a substantial proportion of white matter cells have not been exchanged for at least 5 decades. (C) The ^{14}C concentration in biochemically isolated myelin from the corpus callosum corresponds to the time around the death (indicated with arrows and dashed line to the respective data points) of the subjects, demonstrating that myelin is contemporary and is exchanged at a high rate. Error bars in (B) and (C) indicate 2 SD.

See also Table S5.

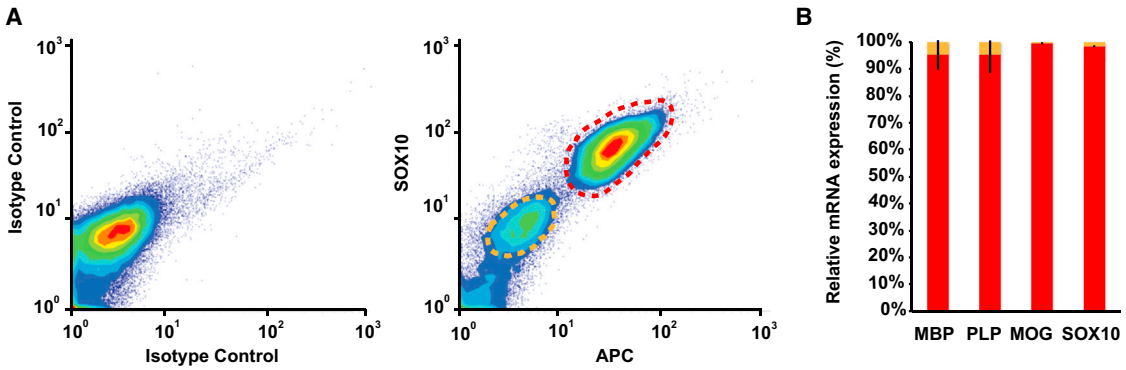

Figure 4. Isolation of Oligodendrocyte Nuclei

(A) Isolation of oligodendrocyte nuclei from the human postmortem corpus callosum by flow cytometry. Labeling with isotype control antibodies (left) and SOX10 and APC antibodies (right) are shown.

(B) Quantitative RT-PCR reveals that almost all of the mRNA for *SOX10* as well as the mature oligodendrocyte markers myelin basic protein (*MBP*), proteolipid protein (*PLP*), and myelin oligodendrocyte glycoprotein (*MOG*) is present in the SOX10+/APC+ nuclei (encircled by red hatched line in [A] and represented by red bar in [B]) and only little of the mRNA is found in the nonoligodendrocyte fraction (marked in orange in [A] and [B]). Data in (B) represent the average from three independent experiments, mean ± SD (n = 3 individuals).

See also Figure S3.

Oligodendrocyte Turnover Dynamics in Human White Matter and Its Relationship to Myelination

With mathematical modeling, it is possible to reproduce the ^{14}C levels that a cell population would have if it followed any pattern of generation and loss and to establish the dynamics of cell generation (Bergmann et al., 2009; Ernst et al., 2014; Spalding et al., 2013) (see Extended Experimental Procedures and Data S1). The ^{14}C data were not compatible with any substantial increase in oligodendrocyte number after 5 years of age (Figure S5A), providing independent validation of the time line for the establishment of the final number of oligodendrocytes (Figure 1C, see Extended Experimental Procedures). Stable oligodendrocyte numbers from the age of five and after that an annual exchange rate of 0.32% (global fitting; median individual turnover 0.37%, 95% confidence interval [CI] [0.18%, 0.81%], Figure S5B; Table S6) provided the best fit for the data (sum of squared errors [SSE] = 1.8×10^4, Figure 6, see Extended Experimental Procedures). A model in which the turnover was restricted to a subpopulation of cells (Ernst et al., 2014), provided a worse fit than models in which all oligodendrocytes were equally likely to be exchanged. We considered the possibility that oligodendrocytes potentially could be generated by direct differentiation of oligodendrocyte progenitor cells, without cell division. The number of oligodendrocyte progenitor cells, and their rather stable numbers (Figure 1B), sets the limit for how much such a process could contribute to oligodendrocyte generation, and we found that this could maximally increase the annual turnover from 0.32% to 0.33% (see the Extended Experimental Procedures). There was no change in the oligodendrocyte turnover rate with age (Pearson's correlation: r = 0.06, p = 0.76, Figure S5B) and no difference in turnover rate between males and females (p = 0.68, Mann-Whitney U test).

After 5 years of age, there was no correlation between oligodendrocyte number and myelin volume (Figure 2B). Moreover, there was no correlation between the oligodendrocyte turnover rate and myelin volume in the corpus callosum (Pearson's correlation, r = 0.23, p = 0.22). Thus, neither the number of oligodendrocytes nor their exchange rate is a major determinant of myelin volume in humans.

Modeling of the ^{14}C data from gray matter oligodendrocytes revealed that the expansion phase is much more prolonged in the cortex compared to white matter and the number of oligodendrocytes does not reach a plateau until the fourth decade of life, with an annual turnover of 2.5% after that. Thus, the kinetics of oligodendrocyte generation and turnover differs between gray and white matter, and it is possible that de novo myelination in the sparsely myelinated cortex may have a role in higher brain functions.

The Oligodendrocyte Number in the Corpus Callosum Is Established in Childhood with Little Influence by Later Generation

When in life is the large interindividual variation in white matter oligodendrocyte number (Figure 1C) established? It could hypothetically be established during the rapid expansion phase until 5 years of age, after this phase, or during both phases (Figure 7A). There is much less interindividual variation in oligodendrocyte numbers before 5 years of age than there is afterward ($p < 0.0001$, two-sample t test, Figure 1C; Table S2; see Extended Experimental Procedures). There is no correlation between the oligodendrocyte generation rate and the number of oligodendrocytes in the corpus callosum (Pearson's correlation: $r = -0.34$, $p = 0.06$) and the ^{14}C data exclude any significant influence of cell exchange after 5 years of age on the number of oligodendrocytes. Together, this implies that the number of oligodendrocytes increases rapidly in a stereotyped manner in early childhood, and the extent of this expansion up to ~5 years of age determines the final complement of oligodendrocytes (Figure 7A). The replacement of 1 in 300 oligodendrocytes per year after that period has little influence on the number of oligodendrocytes and may serve to maintain the constant number of oligodendrocytes. With the limited turnover of white matter

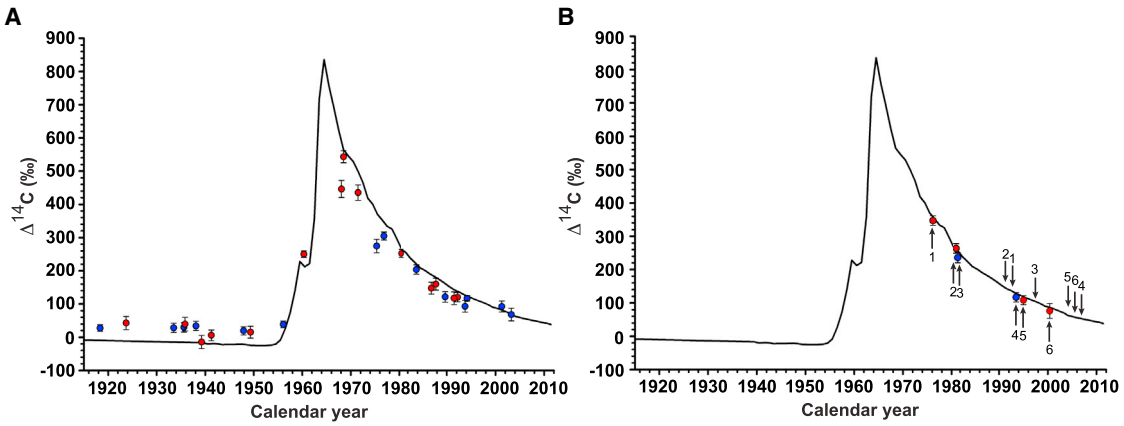

Figure 5. Birth Dating Oligodendrocytes

^{14}C concentrations in genomic DNA of SOX10+/APC+ oligodendrocytes demonstrate limited cell turnover in subjects who died 2009–2012 (A) and in archival specimen from individuals (1-6, arrows below the ^{14}C curve) with earlier death dates (date of death, 1-6, arrows above the ^{14}C curve) (B). Males are indicated in blue and females in red. Error bars show 2 SD.

See also Figure S4 and Table S5.

oligodendrocytes in humans, less than one-third of these cells will be exchanged even during a very long life (Figure 7B).

DISCUSSION

Neural plasticity is key to learning and adapting to novel environments. Most research on neural plasticity has focused on the neurons per se, but an increasing number of observations indicate that modulation of myelination may contribute to neural plasticity by optimizing the performance of the neuronal circuitry. Studies in experimental animals have suggested that oligodendrocyte turnover is an important component of myelin plasticity (Gibson et al., 2014; Young et al., 2013). We report that the oligodendrocyte population in humans is much more static than in previously studied mammals, and oligodendrocyte turnover cannot account for the observed white matter plasticity in response to external cues in humans.

Different Oligodendrocyte Generation Rates in Mice and Humans

The generation of oligodendrocytes has been assessed in quite some detail in the mouse white matter and genetic fate mapping of oligodendrocyte progenitor cells in transgenic mice has yielded quantitative insight into this process (Rivers et al., 2008; Young et al., 2013; Zhu et al., 2011).The oligodendrocyte generation rate in humans (0.32%/year, constant from 5 years of age) is at least 100-fold lower compared to mice (36.5%–182%/year, during adolescent and adult stages, see Extended Experimental Procedures). Oligodendrocyte generation is more pronounced in gray than white matter in humans, but gray matter oligodendrogenesis has not been studied in detail in rodents, precluding comparisons between species. It is possible that the comparatively longer period of growth of the rodent compared to the human brain contributes to a higher generation rate of oligodendrocytes in the mouse white matter. However, there is not a general difference in cell turnover in the brain between these species, as for example the turnover rate of hippocampal neurons, which mediate another type of neural plasticity, is similar in mice and humans (Spalding et al., 2013).

White Matter Volume Is Much More Dynamic Than the Oligodendrocyte Population in Humans

Establishing the dynamics of oligodendrocyte generation allows us to relate this process to the white matter changes in response to experience. A task that requires learning, such as practicing juggling, can in 6 weeks result in a 5% increase in white matter fractional anisotropy (Scholz et al., 2009), an imaging parameter correlating with myelin volume (Blumenfeld-Katzir et al., 2011; Sampaio-Baptista et al., 2013). Thus, myelination is very much more dynamic than oligodendrocyte generation in human white matter and myelin generated by new oligodendrocytes cannot account for the observed magnitude of white matter plasticity in response to a learning situation. Instead, myelination may be modulated by preexisting oligodendrocytes in humans. It will be interesting to assess myelin sheath thickness and axon diameters during development and in adulthood in humans, as these are important parameters that may modulate the function of the neural circuitry (Fields, 2013), but these types of analyses are technically very challenging in human postmortem tissue.

We cannot exclude that myelin remodeling in humans may be carried out by oligodendrocyte turnover in restricted domains of for example the corpus callosum. However, if that is the case, these regions must be very small. If the turnover rate in a restricted region would be for example 3%/year (corresponding to a tenth of lowest estimate in the mouse), then such a region could constitute maximally 10% of the corpus callosum and it would not allow for any oligodendrocyte exchange in the remaining corpus callosum. There is quite substantial interindividual variation in the number of oligodendrocytes as well as myelin volume in humans, posing the question whether oligodendrocyte turnover could affect myelin remodeling to a substantial degree in some individuals. However, this appears unlikely as the

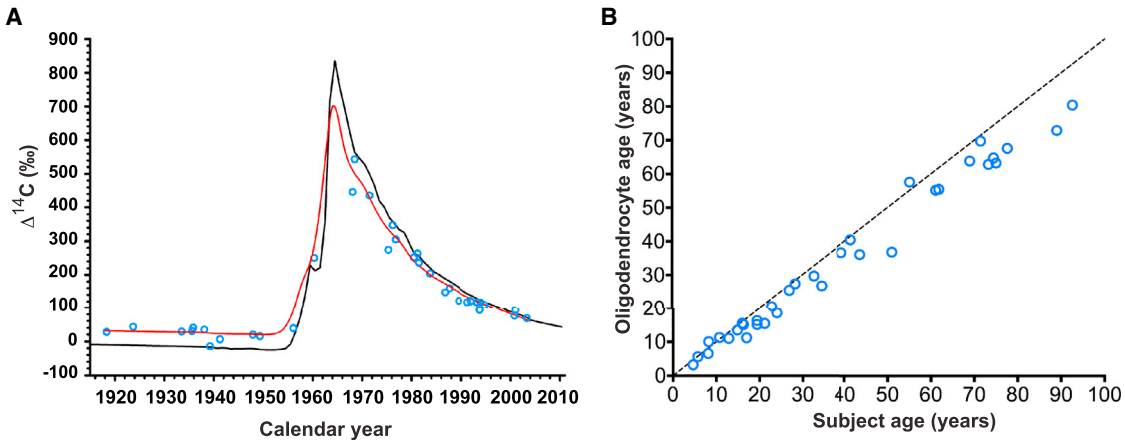

Figure 6. Oligodendrocyte Turnover Dynamics in Humans

(A) Mathematical modeling reveals that the ^{14}C incorporation in oligodendrocyte genomic DNA is best explained by a stable turnover rate of 0.32% per year after 5 years of age, without any substantial increase in cell number. ^{14}C data points are shown as blue circles, values deduced by the model are depicted by red line. (B) With the limited exchange of oligodendrocytes, the average age of this population is only a few years younger than the subject. The hatched line indicates the no turnover scenario.

See also Figure S5 and Table S6.

interindividual variation in oligodendrocyte number is established in early childhood, and after this period, there is no correlation between the turnover and the number of oligodendrocytes.

Mechanisms of White Matter Plasticity

Do humans and rodents utilize altogether different mechanisms for myelin remodeling? Our data establish that oligodendrocyte generation does not play a major role in myelin remodeling in humans, in contrast to rodents. However, although it has been demonstrated that oligodendrocyte generation contributes to myelin remodeling in rodents, this does not exclude that also mature oligodendrocytes may modulate their myelination in rodents. Even though an oligodendrocyte's myelin sheaths may be established short after differentiation (Czopka et al., 2013; Watkins et al., 2008), it is possible that the thickness of the myelin may be altered. Genetic ablation of *PTEN* in oligodendrocytes in mice results in thicker myelin sheaths, indeed indicating the possibility to modulate myelin sheath thickness by mature oligodendrocytes in rodents (Goebbels et al., 2010; Snaidero et al., 2014).

Exchanging oligodendrocytes likely comes at a cost in terms of neurological function, as it appears inevitable that axons become focally demyelinated and unable to properly transmit signals efficiently in the time between the removal of an old oligodendrocyte and its myelin sheaths until the axons are remyelinated by a new cell. A static oligodendrocyte population with the ability to remodel its myelination may have evolved to enable more efficient neural plasticity.

EXPERIMENTAL PROCEDURES

Tissue Collection

Tissues were procured from cases admitted for autopsy at the Department of Forensic Medicine in Stockholm 2009–2012, after receiving consent from relatives. Ethical permission for this study was granted by the Regional Ethics Committee of Sweden (02-418, 2005/185, 2005/1029-31/2, 2006/189-31/1, 2010/313-31/3). White matter tissue samples from different regions (frontal white matter and corpus callosum) were dissected and adjacent gray matter was carefully removed, prefrontal cortex (gray matter and adjacent white matter were dissected and separated) and control tissue from occipital cortex and cerebellar cortex were dissected and stored at −80°C until further analysis. Corpus callosum, prefrontal and frontal cortex specimens from pediatric subjects were obtained from the National Institute of Child, Health, and Human Development (NICHD) Brain and Tissue Bank for Developmental Disorders at the University of Maryland, with ethical permission granted by the institutional review boards of the University of Maryland. Formalin-fixed and paraffin-embedded cortical tissue sections from frontal and occipital lobe were obtained from cancer patients who had received IdU as a radiosensitizer for therapeutic purposes at the National Heart, Lung and Blood Institute, NIH.

Myelin Isolation and Volume Measurement

Before analysis, the whole collected samples of corpus callosum were weighed. For each measurement, samples from all regions of corpus callosum were sampled and 1–8 g of tissue was used. The crude myelin fraction was collected, formed as a top layer of the supernatant, after homogenization and centrifugation (see nuclei isolation) and further processed for volume measurement analysis. The collected crude myelin fraction was resuspended in 10 ml cold Tris-Cl buffer solution (20 mM Tris-HCl [pH 7.45], 2 mM EDTA, 1 mM DTT) and 10 ml of cold 1.8 M sucrose solution (1.8 M sucrose, 3 mM magnesium acetate, 1 mM DTT, 10 mM Tris-HCl [pH 8.0]). The solution was equally layered onto a cushion of 1.5 ml of 1.8 M sucrose solution into two tubes (13.2 ml Thinwall, Ultra-Clear tube, Beckman Coulter) and overlayered with 1 ml of 0.32 M sucrose solution (0.32 M sucrose, 20 mM Tris-HCl [pH7.45], 2 mM EDTA, 1 mM DTT), and centrifuged at 26,500 × *g* for 45 min at 4°C (JS13.1 rotor, Avanti J-26S). Pictures of the tubes with the myelin bands together with a ruler used as a scalebar were taken (Canon PowerShot S100) and a minimum of four pictures per tube were analyzed. The myelin band thickness was analyzed in ImageJ and the volume was calculated by the formula for the volume of a cylinder: $V = h \times \pi \times r^2$. The measured myelin volume was normalized to myelin volume per gram corpus callosum and the total myelin volume in corpus callosum was established by calculating the total weight of the corpus callosum and the myelin volume per gram.

Nuclear Isolation

Tissue samples were thawed and homogenized with a glass Douncer. For each ^{14}C measurement ~8 g of tissue was used and homogenized in 80 ml

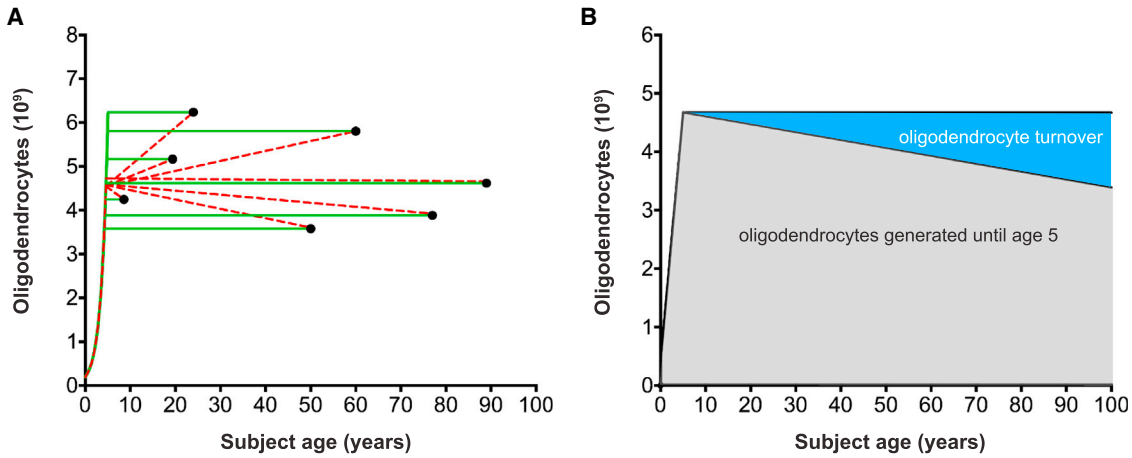

Figure 7. Time Line for Oligodendrocyte Generation in Humans

(A) There is no correlation between the turnover of oligodendrocytes and the oligodendrocyte number, and the data exclude any major change in oligodendrocyte number after 5 years of age (indicated with red hatched lines for a subset of data points). The number of oligodendrocytes is determined ~5 years of age and stays constant thereafter (green lines).

(B) Representation of the population of oligodendrocytes that have been generated until 5 years of age (gray) and the proportion that is exchanged with time (blue). The figure is based on the ^{14}C data, but constrained by the stereological data to also be in accordance with the cell number development, see Extended Experimental Procedures.

ice cold lysis buffer (0.32 M sucrose, 5 mM $CaCl_2$, 3 mM magnesium acetate, 2.0 mM EDTA, 10 mM Tris-HCl [pH 8.0], 0.1% Triton X-100, 1 mM DTT). The homogenized tissue solution was suspended in 160 ml of ice cold 1.8 M sucrose solution. The solution was equally layered onto a cushion of 10 ml 1.8 M sucrose solution into eight tubes and centrifuged at 26,500 × *g* for 2 hr at 4°C (JS13.1 rotor, Avanti J-26S). The supernatant was carefully discarded and the pellet in each tube was resuspended with 1.5 ml of nuclei storage buffer (0.43 M [15%] sucrose, 70 mM KCl, 2 mM $MgCl_2$, 10 mM Tris-HCl [pH 7.2]) for flow cytometry analysis.

Flow Cytometry

Isolated cell nuclei were incubated with primary antibodies against SOX10 (1:250, goat, R&D) and APC (1:250, mouse, clone CC-1, Abcam), or isotype control antibodies for 1 hr on ice. The nuclei solution was washed using nuclei storage buffer and centrifuged at 200 × *g* for 3 min. Species-specific fluorophore-conjugated secondary antibodies (Alexa Fluor 488 and 647, 1:1,000, Molecular Probes, Invitrogen) were added and incubated on ice for 1 hr and thereafter washed with nuclei storage buffer. Single nuclei were separated from doublets, triplets, or higher-order aggregated by a gating strategy using physical parameters as previously described (Spalding et al., 2005). For isolation of all cell types (Figure 3), unlabeled nuclei were collected in the flow cytometer. For isolation of cortical and cerebellar neurons, NeuN (1:1,000, mouse, clone A60, Chemicon) antibody was directly conjugated to Alexa Fluor 647 (Alexa Fluor 647 Antibody Labeling Kit, Invitrogen) and used. The purity of the sorted nuclei was ensured by reanalyzing the sorted populations. The nuclei pellets were collected by centrifugation at 1,500 × *g* for 10 min and further processed for DNA extraction. Flow cytometry analyses and sorting were performed using FACSVantage DiVa, Influx and ARIA flow cytometers (BD Bioscience).

Mathematical Modeling

In the mathematical analysis of the data we used statistical regression and partial differential equations (PDE). The different regression models were used to describe how the cell number changes with age and to guide the modeling of cell birth and death processes with the PDE. The parameters of main interest are the change in cell number, the birth rate, and the death rate. They determine the distribution of cell ages in the population, which is the model component that is integrated with the atmospheric bomb curve. This integration gives the average ^{14}C concentration in the population. Consequently, the modeling consisted of finding the parameters that resulted in an average ^{14}C that is as close as possible to the measured. This was performed with a nonlinear least square algorithm in MATLAB R2011b, which minimizes the squared errors between measured and fitted ^{14}C values. We evaluated the model by investigating the possible correlation between turnover rate and subject age because this would indicate if other models, with nonconstant turnover, needed to be evaluated. No such correlation was found, and we concluded that the selected model best described the oligodendrocyte renewal. The details of the above are included in the Extended Experimental Procedures and in Bergmann et al. (2009).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, five figures, one data file, and six tables and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2014.10.011.

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